Effects of PCB on Reproductive Success in Sprague-Dawley Rats Exposed to Aroclor 1254® for One Year[1]

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ABSTRACT. Polychlorinated biphenyls (PCB) are environmental contaminants that have been a problem since the 1960s. PCB are a serious concern because they are widespread, lipophilic, and bioaccumulate through food webs concentrating in adipose tissue. PCB are a threat to both wildlife and humans because they elicit metabolic and endocrine disruptions with some problems including hypothyroxinemia, spatial learning and memory deficits, neurochemical and neurobehavioral alterations, and reproductive alterations. Sprague-Dawley rats were obtained that had been exposed to Aroclor 1254® in the diet at 1.25 ppm or 25.0 ppm dosage for one year before being mated. Males and females were placed together in a cage and females were tested each day for a sperm-positive vaginal smear. After a sperm positive vaginal smear, females were housed separately, maintained on their respective diets, and weighed daily until weight gain reversal or miscarriage. All animals in the present study that were fed PCB had weight gain reversal or miscarriage, whereas controls of a similar age all carried pregnancy to term. The results of the present study indicate that continuous ingestion of Aroclor 1254® at low doses has a depressive effect on reproductive success.

OHIO J SCI 102 (5):102–105, 2002

INTRODUCTION

Polychlorinated biphenyls (PCB) are environmental contaminants that have been a recognized problem since the 1960s (Jacobsen and Jacobsen 1997). PCB are a serious concern because they are widespread, lipophilic, and bioaccumulate through food webs by concentrating in adipose tissue. These contaminants are a threat to both wildlife and humans because they cause metabolic and endocrine disruption (Aronson and others 2000; Gerstenberger and others 2000). Some of the problems associated with PCB include hypothyroxinemia, spatial learning and memory deficits, neurochemical and neurobehavioral alterations, and reproductive alterations (Schantz and others 1997). Accidental ingestion by humans, which occurred in Japan in 1968 and Taiwan in 1979, has caused many long-term effects including hyperpigmentation, inflamed conjunctival glands, and intellectual impairment (Kuratsune and others 1972). As a result of human exposure, PCB have been detected in human serum, adipose tissues, breast tissues, and brain tissues (Aronson and others 2000).

Previous studies done in our lab using large amounts of the PCB mixture Aroclor 1254® (62.5, 125, or 250 ppm) in the maternal diet have reported a decrease in body weight, spatial learning problems, and subnormal thyroid status in young rats (Juárez de Ku 1992; Pritts 1996). Using smaller amounts of Aroclor 1254® (1.25, 12.5, or 25.0 ppm) resulted in alterations in pup thyroid status, depressed body weight, and problems in spatial learning in the Morris Water Maze (Pritts 1996; Provost and others 1999). The present study was designed to examine the long-term reproductive effects of Aroclor 1254® on animals exposed to a diet containing small amounts of PCB (1.25 and 25.0 ppm) and on the second generation of offspring from animals exposed for one year.

MATERIALS AND METHODS

Sprague-Dawley rats were obtained from the Bowling Green State University Animal Research Facility. Animals received food and water ad libitum in a temperature-monitored environment of 20° C and 20-50% humidity. Parents of the animals used in the present study were female rats weighing 225-275 g that were mated to males of the same strain. Once females were determined to be pregnant, confirmed by a sperm-positive vaginal smear, they were caged separately. Rats were fed a diet consisting of standard rat chow (Mowlan Teklad, Madison, WI) with Aroclor 1254® (AccuStandard, Inc., New Haven, CT) added at 1.25 or 25.0 ppm (w/w). Control animals were continued on standard diet after conception. On the third day following birth, litters were culled to eight pups consisting of four females and four males, where possible. The pups remained in the maternal cage until thirty days of age, at which time two rats were removed for use in another study. The remaining six rats were housed three females and three males per cage, where possible.

Ten of the rats for the present study were maintained on the control diet of standard rat chow. Six of the rats for the present study were born to mothers fed PCB, and were given a control diet (consisting of standard rat chow with no PCB) at thirty days of age. The remaining thirty rats were left on the PCB-containing diet that their mothers were fed from the first day of pregnancy (standard rats chow containing Aroclor 1254® at 1.25 or 25.0 ppm). At approximately one year of age these female rats were mated to males of the same strain with varying degrees of exposure to PCB. The mating protocol resulted

[1]Manuscript received 9 October 2001 and in revised form 20 March 2002 (#01-26).

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in the generation of a total of five groups. Controls consisted of mating pairs of female and male rats that had never been exposed to PCB. Treatment group one consisted of PCB-control males (males exposed to Aroclor 1254® during gestation and for thirty days after birth, then given a control diet) mated to 1.25 ppm Aroclor 1254® females. Treatment group 2 consisted of PCB-control males mated to 25.0 ppm Aroclor 1254® females. The 3rd treatment group consisted of males and females both continuously exposed to 1.25 ppm Aroclor 1254® from gestation. The 4th treatment group consisted of males and females continuously exposed to 25.0 ppm Aroclor 1254® from gestation. Females pregnant from these matings were caged separately and weighed daily until birth of pups or miscarriage. Statistical analysis was performed using StatView (SAS Institute, Cary, NC). Statistical significance was ascribed to $p < 0.05$ with ANOVA and Fisher's PLSO was used to detect statistical differences between groups.

RESULTS

All PCB-fed animals demonstrated an increase in the time between placement of males and females in the same cage and presence of a sperm-positive vaginal smear (Fig. 1), as compared with controls ($p < 0.001$). Animals exposed to 25.0 ppm Aroclor 1254® required a significantly greater time of cohabitation for copulation to occur than either controls or 1.25 ppm animals ($p < 0.007$) (Fig. 1).

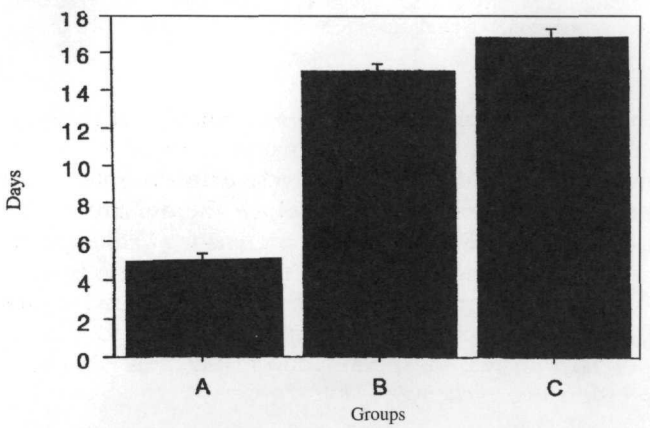

FIGURE 1. Time to presence of sperm-positive vaginal smear (A = Controls, 5.0 ± 0.3; B = 1.25 ppm PCB, 15.0 ± 0.4; C = 25 ppm PCB, 16.8 ± 0.2). Animals that were exposed to PCB had significantly greater time of cohabitation for copulation to occur than controls ($p < 0.001$).

All control females delivered normal, healthy young. None of the PCB-exposed groups produced young, with pregnancy ending in miscarriage ($p < 0.001$) (Fig. 2). Miscarriages were detected by reversal of weight gain demonstrated by all females after a sperm-positive vaginal smear (Fig. 3). When an animal returned to its pre-pregnancy weight or less, vaginal smears were performed in order to determine whether reproductive cyclicity had been re-established, and return to cyclicity was taken as evidence of miscarriage. All animals which were assumed to have miscarried returned to reproductive cyclicity by what would have been the middle of pregnancy (day 11) (Fig. 3).

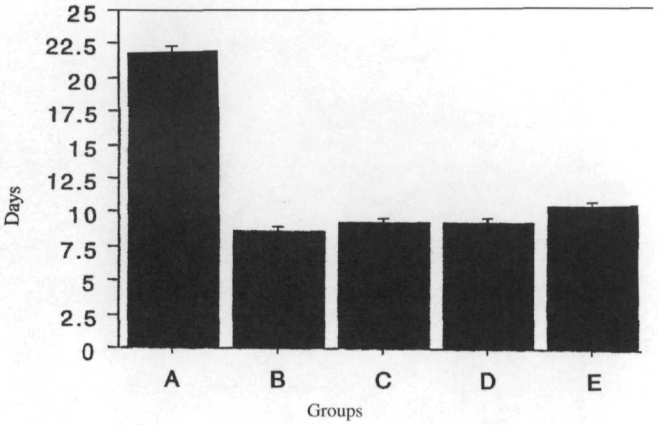

FIGURE 2. Length of pregnancy maintenance in controls and rats exposed to PCB from conception (A = Controls, 21.8 ± 0.3; B = PCB-Control males mated to 1.25 ppm PCB females, 8.7 ± 0.5; C = PCB-Control males mated to 25.0 ppm PCB females, 9.3 ± 0.7; D = 1.25 ppm PCB males mated to 1.25 ppm PCB females, 9.4 ± 0.3; E = 25.0 ppm PCB males mated to 25.0 ppm PCB females, 10.7 ± 0.5) ($p < 0.001$). All PCB-exposed animals failed to produce viable young, with miscarriage occurring around day 9.

DISCUSSION

The results of the present study indicate that Aroclor 1254® has a depressive effect on reproductive success in animals exposed to this PCB mixture for one year. The effect of Aroclor 1254® may be explained if one examines more closely reported effects of PCB on reproduction.

Polychlorinated biphenyls have been shown to mimic estrogenic affects (Ahmed 2000) by actively competing for estrogen receptors (Krogenaes and others 1998) and by causing modifications similar to those resulting from an overproduction of estrogen (Safe 1994). Ovulation in rats occurs during estrus temporally coincident with behavioral changes that allow copulation to occur (Hadley 2000). An explanation for the increase in time to copulation for animals exposed to Aroclor 1254® for one year may be based on occurrence of delays of behavioral estrus of the animals. Jonsson and others (1976) exposed animals to Aroclor 1242® in dosages of 3.7 and 7.5 mg/kg/day (equivalent to 111 ppm and 225 ppm) for 36 weeks and observed altered estrous cycles, most often skipping estrus, with ovulation inhibited in 50% of treated animals. Brezner and others (1984) showed that in rats exposed to 10.0 mg/kg/day (equivalent to 300 ppm) of Aroclor 1254® for 6 weeks, the animals developed prolonged estrous cycles. These previous investigators used large doses of PCB to cause altered estrous cycles, which would have increased time to copulation. Although cycle length was not measured in the present study, demonstrated increased time to copulation may be inferred that the estrous cycle was altered. Previous studies have treated animals with PCB for 0-6 months, but never for longer than 6 months. The present study treated the animals with PCB for one year, and the possibility exists

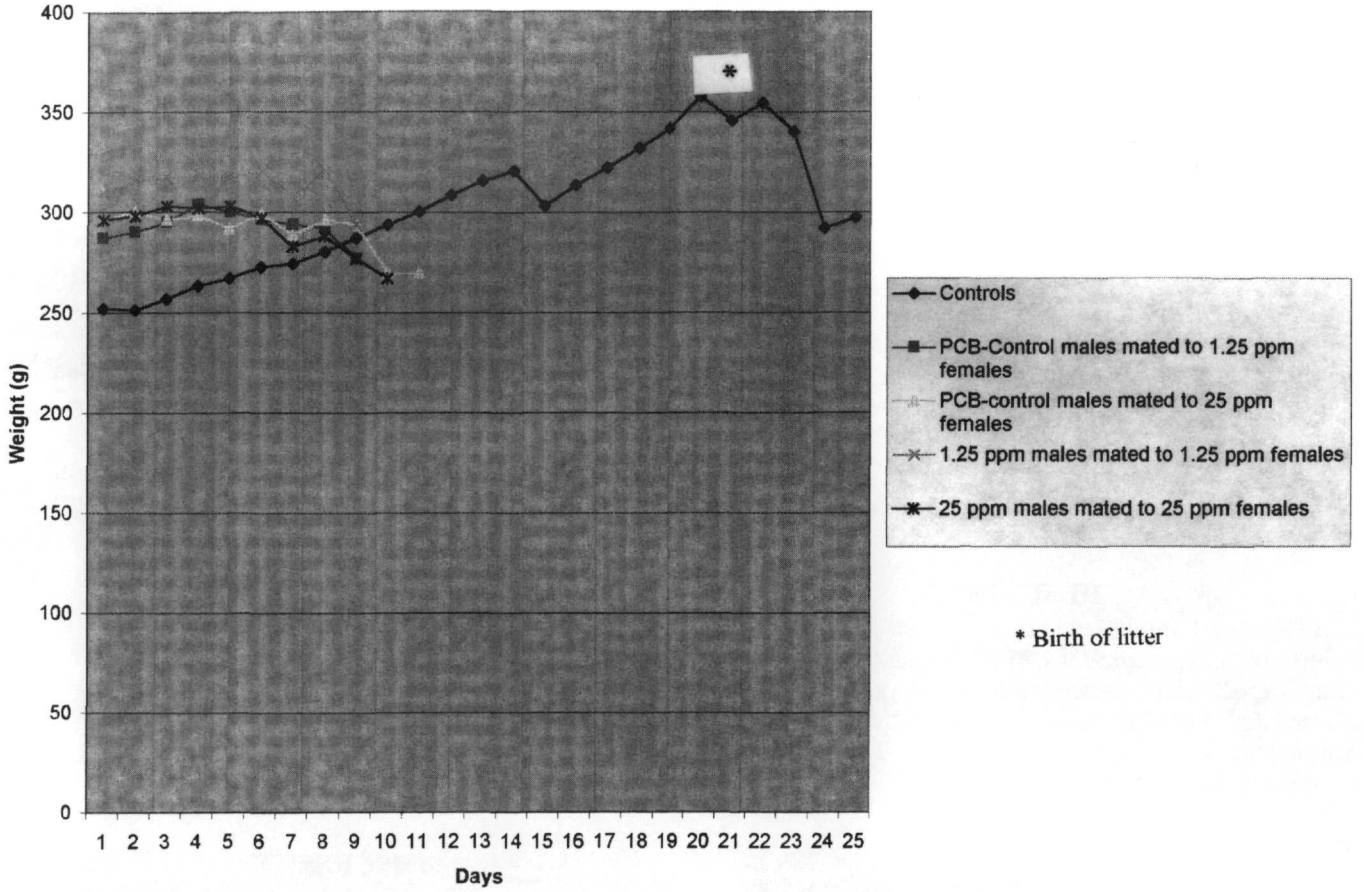

FIGURE 3. Weight gains and losses of control and PCB-exposed animals. Weight gains from days 1-6 of pregnancy in PCB-treated animals were reversed in all groups by day 7 ($p < 0.001$). Error bars were not included to avoid confusion, but in all cases SEM was less than 10% of the mean.

that bioaccumulation of the smaller dosages of PCB used in the present study (1.25 or 25.0 ppm) may be equal to the short duration of larger dosages of PCB.

Since all males in the present study were also exposed to PCB, their input to unsuccessful pregnancy maintenance can also be considered. Rats undergo fertilization by oogamy, where mobile sperm swim to a stationary egg (Gilbert 2000). It has been found that in male animals exposed to PCB, doses as small as 1.25 ppm have altered semen quality (Guo and others 2000). The same study reported that humans exposed to PCB have reduced daily sperm production and an increase in the number of abnormal sperm. PCB have been reported to cause alterations in sperm, including mutation in the genome and outward phenotypic expression (Guo and others 2000). Sager and others (1987) reported that early postnatal exposure to Aroclor 1254® did not affect the production, morphology, or motility of sperm; however, the ability of these sperm to fertilize eggs was severely impaired. If the sperm were able to swim to the egg and successfully fertilize the egg, it is possible that the zygote that resulted was abnormal, which might cause the natural abortion of the young by our exposed animals.

Future studies should include breeding of unexposed males to PCB-exposed females and also breeding unexposed females to PCB-exposed males. This would help to determine whether the reproductive effects seen in exposed animals in the present study were caused by the males, females, or both sexes. Krajnak and others (2001) showed that, as an animal ages, there is a delay in completion of the estrous cycle extending the cycle beyond the normal 4-5 days. Since the animals in the present study were one year of age, mating PCB-exposed rats at 6-12 months of age may allow for viable offspring. With such offspring, it might be possible to investigate the specific mechanisms that come into play to explain potential increased sperm abnormality and abnormal reproductive cyclicity.

ACKNOWLEDGMENTS. The authors thank Dr. Suzanne K. Miller for her financial assistance; Denise Hook, KoriAnne Bagrowski, Brent Drouillard, and the BGSU Animal Care Facility for their help in raising the animals; Anne Collaco for her help with StatView and the endless questions concerning the program; and Dr. Stan L. Smith for his comments on this manuscript.

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